

**(1) Wording informalities in the claims objection**

Claims 24-43 are cancelled. Newly presented claims incorporate the Examiner's instructions in regard to earlier wording informalities.

**(2) 35 USC § 112, first paragraph – Written Description**

Claims 24-31 and 33-43 are cancelled. New claims 44-45, 53-54, 62-63, and 71-72 are proposed to further define allowed claim 23. They are directed to materials described in the specification on pages 13-14 (ref. new claims 44-45, 53-54, and 62-63), and beginning in the last paragraph on page 23 continuing through page 25 (ref. claims 71-72).

New claims 46-52, 55-61, 64-70, and 73-79 narrow the content to cover only claims for which direct experimental results are described in the specification. Experimental evidence describing root aerenchyma (ref: new claims 46, 55, 64, 73) is presented in the specification beginning page 26, last paragraph, through the top of page 28. Experimental evidence describing drought tolerance (ref. new claims 47, 56, 65, 74) is presented in the specification in the first paragraph on page 28. Experimental evidence describing corn rootworm tolerance (ref. new claims 48, 57, 66, 75) is presented in the specification in paragraph 2, page 24 continuing on page 25. Experimental evidence describing SSR markers bnlg 2235, dupSSR23, phi123, and bnlg 1714 (ref. new claims 49-51, 58-60, 67-69, 76-78) is described in the specification beginning last paragraph on page 25 continuing on page 26. Experimental evidence describing SSR marker bnlg1805 (ref. new claims 52, 61, 70, 79) is described beginning last paragraph page 28 continuing on page 29.

Please be advised there is a typographical error on page 29 of the specification in the sentence beginning on line 3 continuing on line 4 that reads as follows: "The corresponding SSR marker for the BNL8.32 locus is bnlg2235." Please correct the sentence to read: "The corresponding SSR marker for the BNL8.32 locus is bnlg1805." The correct information is provided elsewhere in the specification on page 52, Table 5, columns 5 and 6, lines 7 under the Chromosome 7 header, and in the 7<sup>th</sup> entry under chromosome 7 in Figure 2.

**(3) 35 USC § 112, first paragraph - Enablement**

Claims 24-31 and 33-43 are cancelled. New claims 44-45, 53-54, 62-63, and 71-72 are proposed to further define allowed claim 23. They are directed to materials described in the specification on pages 13-14 (ref. new claims 44-45, 53-54, and 62-63), and beginning in the last paragraph on page 23 continuing through page 25 (ref. claims 71-72).

One of ordinary skill in the art can utilize the association genetics method based on the novel alleles presented in claim 23 to determine specific molecular marker locus/loci that is/are associated with a particular phenotypic trait in a segregating population of maize plants introgressed by a *Tripsacum*-teosinte source by: (1) growing out a segregating population of maize plants introgressed by a *Tripsacum*-teosinte recombinant; (2) screening the genomic DNA of the plants using the 138 RFLP-restriction enzyme combinations provided in claim 23; (3) comparing the restriction fragments to the ones listed in claim 23, and (4) determining which marker locus/loci is/are found in all the plants expressing the trait of interest that is/are not found in the sibling plants not expressing the trait of interest. This provides a new, easy, straightforward method of associative genetics enabled by the unique restriction fragments formed in teosinte-*Tripsacum* recombinants that are not found in unintrogressed maize plants. It eliminates molecular marker locus mapping of large populations of maize plants over multiple generations and the requisite complex statistical analyses to determine association of maize molecular marker loci with traits of interest. When one of ordinary skill in the art has determined the association of one or more markers with a trait, they can screen plant seedlings, seed, pollen, or any plant part for the presence of one or more alleles at that marker locus this is/are associated with the trait of interest. This permits early, rapid selection of plants with a trait of interest. Corn rootworm tolerance (described beginning last paragraph on page 24 continuing on page 25 of the specification) provides a working exemplar of how to employ this method to characterize a polygenic trait. Root aerenchyma (described beginning last paragraph on page 26 continuing to page 29) provides a working exemplar of how to employ this method to characterize a single locus trait. These two examples precisely illustrate how one of ordinary skill in the art can employ the method to determine the association of precise marker loci restriction fragments with a trait of interest by comparing the DNA profiles of plants of the same genotype in which some plants exhibit the trait of interest and some plants do not.

New claims 46-52, 55- 61, 64-70, and 73-79 narrow the content to cover only claims for which direct experimental results are described in the specification. Experimental evidence describing root aerenchyma (ref. new claims 46, 55, 64, 73) is presented in the specification beginning page 26, last paragraph, through the top of page 28. Experimental evidence describing drought tolerance (ref. new claims 47, 56, 65, 74) is presented in the specification in the first paragraph on page 28. Experimental evidence describing corn rootworm tolerance (ref. new claims 48, 57, 66, 75) is presented in the specification in paragraph 2, page 24 continuing on page 25. Experimental evidence describing SSR markers bnlg 2235, dupSSR23, phi123, and bnlg 1714 (ref. new claims 49-51, 58-60, 67-69, 76-78) is described in the specification beginning last paragraph on page 25 continuing on page 26. Experimental evidence describing specific describing SSR marker bnlg1805 (ref. new claims 61, 70, 79) is described beginning last paragraph page 28 continuing on page 29.

#### (4) 35 USC § 102(b)

Claims 24-27, which were rejected under 35 U.S.C. 102(b) as being anticipated by Eubanks (U.S. Patent No. 5,330,547, 1994) and Eubanks (U.S. Patent No. 5,750,828, 1998), are cancelled. In accordance with the discussion about how to write product claims in the September 2007 the patent office interview, newly proposed claims 44-45, 53-54, and 62-63 are directed specifically to crosses, backcrosses, and materials that require steps not described or claimed in Eubanks (U.S. Patent No. 5,330,547, 1994 and U.S. Patent No. 5,750,828, 1998). The way to make the new types of crosses and backcrosses that produce the products of claims 44-45, 53-54, and 62-63 is described on pages 13-14 of the specification. Said crosses and backcrosses were not mentioned or described in any earlier patents or publications. Therefore, neither Eubanks (U.S. Patent No. 5,330,547, 1994), nor Eubanks (U.S. Patent No. 5,750,828, 1998), anticipated the new product claims.

Some specific examples of said crosses and backcrosses are a (*Tripsacum*-teosinte female X maize male) designated TC64 in Tables 2 and 3 and on page 22 of the specification, and a (*Tripsacum*-teosinte female X maize male) backcrossed to a *Tripsacum*-teosinte recombinant, designated 7022 in Tables 2 and 3 and on page 22 of the specification.

**(5) 35 USC § 102/103**

Claims 28-43, which the Examiner rejected "under 35 U.S.C. 102(b) by, or in the alternative, under 35 U.S.C. 103(a) as obvious over Eubanks (U.S. Patent No. 5,750,828, 1998), taken with the evidence of Smith et al. (Theor. Appl. Genet. 95:163-173, 1997)," are cancelled. Newly proposed claims such that "the invention is not identically disclosed or described as set forth in section 102," are claims 46-52, 55-61, 64-70, and 73-79.

Based on the Smith et al. reference (Theor. Appl. Genet. 95:163-173, 1997) cited in the 29 August 2007 Office Action, the Examiner wrote: "One of ordinary skill in the art, at the time of the filing of the application, would have known that markers were available to identify maize plants with various traits because Smith et al. teach SSR markers in maize to characterize and identify maize inbred lines, validate pedigree, and show associations among inbred lines" (29 August 2007 Office Action, page 10, 3<sup>rd</sup> paragraph, lines 3-7). Smith et al.'s findings from comparative study of SSR and RFLP markers in maize underscore that one of ordinary skill in the art would not have anticipated the invention of this application as obvious over Eubanks (U.S. Patent No. 5,750,828, 1998). The basic principle of Mendelian inheritance that one of ordinary skill in the art would expect is that the progeny of maize lines would inherit one allele for a trait of interest from one parent and another allele for the same trait of interest from the other parent. If the DNA fingerprint of an offspring exhibits a single band at the molecular locus for the trait of interest being genotyped, the individual is homozygous for a single allele at that particular locus and the assumption is that both parents carry the same allele for that particular trait of interest. If the offspring's molecular fingerprint exhibits two bands at the locus in question, then it has inherited one allele for the trait of interest from one parent and a different allele from the other parent such that the individual's genotype is heterozygous at that locus. Smith et al. characterized 131 SSR loci in 62 maize lines to investigate the frequency of non-Mendelian inheritance, defined as absence of a parental band or presence of a non-parental band (Theor. Appl. Genet. 95:166, 1997). Out of the 131 SSR markers employed, 25 had incidences of "non-Mendelian" scoring, but only one of those was attributed to the presence of a new band in the progeny that was not present in either parent. One of ordinary skill in the art would therefore infer since 1 out of 131 markers detected a non-parental band that a new non-parental band would occur 0.76% of the time. Of the 188 markers Eubanks employed to screen the teosinte-*Tripsacum* introgressed maize lines, one of ordinary skill in the art would predict 1.4

new non-parental bands would occur ( $188 \times 0.0076$ ). The 372 new, non-parental bands described exceed the predicted 1.4 non-parental bands by a factor of 266! Such dramatically unexpected results could not have been predicted nor anticipated based on prior art. The findings of Smith et al. strongly underscore how unexpected and unprecedented the discovery of so many recurring non-parental bands is. The fact that many of the same bands recur in progeny derived from crosses between different parents of the two genera, and that those same novel bands are stably inherited in subsequent generations and various permutations of different teosinte-*Tripsacum* introgressed maize crosses and backcrosses is even more surprising and unpredictable. This is why the novel alleles upon which the claims are based would not have been anticipated by the prior art. The findings defy the basic tenets upon which maize molecular marker mapping, DNA fingerprinting, and genotype analysis are based. Although one of ordinary skill in the art would know how to make crosses and screen with molecular markers, it would NOT have been prima facie obvious to one of ordinary skill in the art to expect to see more than one or two novel, non-parental bands in the DNA fingerprints of plants screened with 188 molecular markers. The results of over 300 novel, non-parental bands are completely unexpected and non-obvious.

In regard to conditions for patentability under 35 U.S.C. § 102/103, according to section 700-19 of the MPEP: “a biological process using or resulting in a composition of matter that is novel under section 102 and non-obvious under subsection (a) shall be considered nonobvious.” If there are unexpected results of two known elements, then the material is patentable because one of ordinary skill of the art would not know that if you put those together you always get that result. One of ordinary skill in the art would not expect to recover a high frequency of novel, non-parental restriction fragments (i.e. that appear from a change in composition of matter) from crosses between *Tripsacum* and teosinte, nor would one of ordinary skill in the art expect the same said non-parental restriction fragments to recur in different crosses with different parent plants, nor that the same said novel restriction fragments would be stably inherited in subsequent generations of crosses and backcrosses with maize.

According to section 16.05 of the MPEP, a *prima facie* case of obviousness is rebutted by incredulity and skepticism of experts. Referring to Eubanks' crosses between teosinte and *Tripsacum*, twelve leading maize geneticists wrote:

First, the chromosome number of the putative *tripsacum*-*diploperennis* hybrid is  $2n = 20$ . This is also the chromosome number of both maize and *Z. diploperennis*, and thus the number one would expect in a maize-*diploperennis* hybrid. If *Tripsacum* ( $2n = 36$  or  $72$ ) had been one of the parents, one would have expected the hybrid to have 28 or 46 chromosomes. Second, the high fertility of the putative *tripsacum*-*diploperennis* hybrid is typical of maize-teosinte hybrids (Wilkes 1967), while the only known teosinte-*tripsacum* hybrids (*T. andersonii*) is sterile (Talbert et al. 1990a). Third, the molecular marker results presented by Eubanks (1995, 1997) do not establish that the putative *tripsacum*-*diploperennis* hybrids are real. Specifically, the putative hybrids do not contain restriction fragments of both of the two putative parents..."

The above quote by Jeff Bennetzen, Edward Buckler, Vicki Chandler, John Doebley, Jane Dorweiler, Brandon Gaut, Michael Freeling, Sarah Hake, Elizabeth Kellogg, R. Scott Poethig, Virginia Walbot, and Susan Wessler appears on page 85, column one, paragraph 2, lines 8-19 continuing in column 2, lines 1-5 of "Genetic Evidence and the Origin of Maize," *Latin American Antiquity* 12:84-86, 2001. These authorities succinctly laid out what one of ordinary skill in the art would predict and how Eubanks' findings differ from what would be expected. This publication by twelve prominent maize geneticists provides robust support against *prima facie* obviousness.

The inherency rule means that prior art **must necessarily** contain the material being claimed. Out of 188 RFLP loci screened, the 138 loci that detect novel restriction fragments not found in either parent reveal a total of 372 new non-parental bands. Of the 372 non-parental restriction fragments described in *Tripsacum*-teosinte recombinants, 251 (67.5%) occur in Tripsacorn, 230 (61.8%) in Sun Star, 200 (53.8%) in Sun Dance, 129 (34.7%) in 20A. Although crosses appear similar, individuals are not genotypically identical and do not necessarily contain any particular non-parental restriction fragment. This is the critical basis of the unique associative genetics method for rapid discovery of precise molecular marker loci associated with particular traits of interest in maize. Through elimination of all the marker loci that exhibit novel alleles in the teosinte-*Tripsacum* introgressed maize progeny that do not express a trait of interest, one of ordinary skill in the art can immediately identify the candidate locus/loci associated with said trait of interest among the remaining marker loci that detect novel alleles in every plant that exhibits said trait of interest. The characterization of the single locus associated with aerenchyma (described in the specification beginning in the last paragraph of page 27, continuing in the last paragraph of page 28 and onto page 29) illustrates how easy and convenient this method is. Two out of three F<sub>1</sub> plants of Tripsacorn X W64a had aerenchyma.

One of two B73 X Tripsacorn plants had intermediate expression of aerenchyma. Because aerenchyma was present only in Tripsacorn and plants designated 2019, 3028 and TC64, and was not present in the other Tripsacorn-introgressed maize progeny (these include 64TC, 4021, 3024, 3028, 3125, 4126, 4029, 7022, 7024, (9094 X 7009), 97-5 X 97-1, and V70 listed on page 22, lines 25-38 of the specification), it was possible to eliminate all other Tripsacorn novel restriction fragments in the plants that did not have aerenchyma and pinpoint the BNL8.32 locus on the long arm of linkage group 7 as the only one associated with inheritance of root aerenchyma.

Please cancel claims 24-43 and enter proposed new claims 44-79. The cancellation of claims 1-22 and 24-43 removes 41 previously entered claims. The proposed new claims 44-79 add back 35 claims, making a total of 36 claims. An excess claims fee of \$475 was paid on 5 September 2006. At \$25 per claim, the excess claim fee covered 19 claims in addition to the 25 claims included in the original filing fee, bringing the total number of claims for which the Applicant has paid to 44. Therefore the current total of 36 entered claims does not require an additional excess claim fee.

Respectfully submitted,



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